## **CLAIMS**

What is claimed is:

1	1. A method for determining the effect of a test agent on a tissue engineered					
2	cartilage matrix, comprising:					
3	(A) culturing an engineered cartilage tissue comprising the steps of:					
4	(i) culturing isolated chondrogenic cells for an amount of time effective					
5	for allowing formation of a chondrogenic cell-associated matrix; and					
<sup>}≟</sup> 6	(ii) culturing the chondrogenic cells with the cell-associated matrix on a					
<b>5</b> 7	semipermeable membrane in the presence of a growth factor for a time effective for allowing					
[∏ _= 8	formation of the engineered cartilage tissue;					
17 17 18 19	(B) contacting one or more test agents with one or more cells or tissues selected					
110	from the group consisting of (a) the isolated chondrogenic cells prior to (i), (b) the chondrogenic					
	cells during (i), (c) the chondrogenic cells and cell-associated matrix prior to (ii), (d) the					
	chondrogenic cells and cell-associated matrix during (ii), and (e) the engineered cartilage tissue;					
112 1113	and					
[] []] 4	(C) measuring the effect the one or more test agents has on the contacted cells or					
15	tissue.					
1	2. The method of claim 1 wherein the chondrogenic cell-associated matrix					
2	comprises aggrecan, collagen types II, IX and XI, matrix proteins and hyaluronan.					
_						
1	3. The method of claim 1 wherein the engineered cartilage tissue comprises					
2	collagen types II, IX and XI, hyaluronan and at least about 5 mg/cc3 aggrecan,					
3	wherein the ratio of aggrecan to hyaluronan is about 10:1 to about 200:1, and the					
<u> </u>	ratio of aggregan to collagen is about 1:1 to about 10:1					



1	4.	The method of claim 1 wherein the isolated chondrogenic cells are from			
2	articular cartilage.				
1	5.	The method of claim 1 wherein the isolated chondrogenic cells are from			
2	costal cartilage, nas	al cartilage, auricular cartilage, tracheal cartilage, epiglottic cartilage, thyroid			
3	cartilage, arytenoid cartilage or cricoid cartilage.				
	3, 3				
1	6.	The method of claim 1 wherein the isolated chondrogenic cells are from			
2	fibrocartilage.				
	S				
1	7.	The method of claim 6 wherein the fibrocartilage is ligament, tendon,			
<b>∔</b> 4 2	meniscus or interver	tebral disc.			
	8.	The method of claim 1 wherein step (i) comprises culturing the			
F 2	chondrogenic cells on an alginate medium.				
<b>F</b> A	_				
[] = 1	9.	The method of claim 1 wherein step (C) comprises measuring the amount			
[] <sub>1</sub> 2	ne engineered cartilage tissue.				
ſΨ					
[ <u>]</u> 1	10.	The method of claim 1 wherein step (C) is performed without the addition			
TU 2	of extrinsic radioact	ivity.			
1	11.	The method of claim 10 wherein step (C) comprises enzymatically			
2	degrading the engin	eered cartilage tissue.			
1	12.	The method of claim 11 wherein step (C) further comprises staining the			
2	enzymatically degra	ded engineered cartilage tissue with a dye.			
1	13.	The method of claim 1 wherein the engineered cartilage tissue is removed			

from the semipermeable membrane prior to being contacted with the test agent.

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1	14.	The method of claim 1 further comprising:				
2	(D) identifying one or more test agents that have desirable properties; and					
3	3 (E) producing the one or more test agents as a therapeutic drug.					
1	15. A	kit for determining the effect of a test agent on a tissue engineered cartilage				
2	2 matrix comprising instructions for carrying out the method of claim 1.					
1	16.	The kit of claim 15 further comprising one or more of:				
2		(i) one or more reagents;				
3		(ii) an enzyme capable of degrading the engineered cartilage tissue;				
4		(iii) a dye capable of labeling a component of the engineered cartilage				
F-4 5	tissue; and					
[] 6		(iv) an antibody capable of labeling a component of the engineered				
<b>1</b> 7	cartilage tissue.					
1 5	17.	A method for determining the effect of a test agent on a tissue engineered				
= 2 []	cartilage matrix, con	nprising:				
[≟ 3	(A)	culturing an engineered cartilage tissue comprising the steps of:				
ry 4		(i) culturing isolated chondrogenic cells for an amount of time effective				
13 4 5 E	for allowing formation of a chondrogenic cell-associated matrix; and					
6		(ii) culturing the chondrogenic cells with the cell-associated matrix on a				
7	semipermeable membrane in the presence of a growth factor for a time effective for allowing					
8	formation of the engineered cartilage tissue;					
9	(B)	contacting one or more test agents with one or more cells or tissues				
10	selected from the group consisting of (a) the isolated chondrogenic cells prior to (i), (b) the					
11	chondrogenic cells during (i), (c) the chondrogenic cells and cell-associated matrix prior to (ii),					
12	(d) the chondrogenic cells and cell-associated matrix during (ii), and (e) the engineered cartilage					
13	tissue in the presence of a known modulator of cartilage tissue; and					
14	(C)	measuring the effect the one or more test agents has on the contacted cells				
15	or tissue.					

1		18.	The method of claim 17 wherein the chondrogenic cell-associated matrix			
2	comprises aggrecan, collagen types II, IX and XI, and hyaluronan.					
1		19.	The method of claim 17 wherein the isolated chondrogenic cells are from			
2	articular cartil	age.				
1			The method of claim 17 wherein the isolated chondrogenic cells are from			
2	costal cartilage, nasal cartilage, auricular cartilage, tracheal cartilage, epiglottic cartilage, thy					
3	cartilage, aryte	enoid ca	artilage or cricoid cartilage.			
		21				
	<b></b>		The method of claim 17 wherein the isolated chondrogenic cells are from			
2	fibrocartilage.					
1		22	The weekle defection 21 who we to the Characteria as is ligament tondon			
1			The method of claim 21 wherein the fibrocartilage is ligament, tendon,			
2	meniscus or ir	iterverte	ebral disc.			
1		22	The method of claim 17 wherein step (i) comprises culturing the			
	.1		• 1/			
2	chondrogenic	cens on	an arginate medium.			
1		24	The method of claim 17 wherein the engineered cartilage tissue comprises			
2	0.0110.00m trm.00		and XI, hyaluronan and at least about 5 mg/cc <sup>3</sup> aggrecan,			
	wherein the ratio of aggrecan to hyaluronan is about 10:1 to about 200:1, and the					
4	ratio of aggree	can to co	ollagen is about 1:1 to about 10:1.			
1		25	The method of claim 17 wherein step (C) comprises measuring the amount			
	of proteoglyce		• • • •			
_	or proteogrycz	ui iii tiic	engineered cartilage tissue.			
1		26.	The method of claim 17 wherein step (C) is performed without the			
2	addition of ex		• • • •			
	2 1 2 1 2 3 1 2 1 2 3 4 1 1 2 1 1 2	2 comprises agg 1 2 articular cartil 2 costal cartilag 3 cartilage, aryte 1 2 fibrocartilage. 1 2 meniscus or ir 1 2 chondrogenic 1 2 collagen types 3 4 ratio of aggree 1 2 of proteoglyca 1	2 comprises aggrecan, of 19. 2 articular cartilage.  1 20. 2 costal cartilage, nasal cartilage, arytenoid cartilage, arytenoid cartilage.  1 21. 2 fibrocartilage.  1 22. 2 meniscus or interverte  1 23. 2 chondrogenic cells on 24. 2 collagen types II, IX arytenoid cartilage arytenoid cartilage.  1 25. 2 of proteoglycan in the 25. 2 of proteoglycan in the 26.			



The method of claim 26 wherein step (C) comprises enzymatically 1 27. 2 degrading the engineered cartilage tissue. The method of claim 27 wherein step (C) further comprises staining the 1 28. 2 enzymatically degraded engineered cartilage tissue with a dye. 29. The method of claim 17 wherein the modulator of the engineered cartilage 1 2 tissue is a matrix stimulating agent, cytokine or TNF-α. 1 30. The method of claim 29 wherein the cytokine is interleukin-1. 31. A kit for determining the effect of a test agent on an engineered cartilage <u>į.</u> 1 口口 口口 1 1 1 2 tissue comprising instructions for carrying out the method of claim 17. 32. The kit of claim 31 further comprising one or more of: (i) one or more reagents; **□** 3 (ii) an enzyme capable of degrading the engineered cartilage tissue; 13 4 14 4 (iii) a dye capable of labeling a component of the engineered cartilage **TU** 5 tissue; and TU 6 (iv) an antibody capable of detecting a component of the engineered N 7 ivcartilage tissue. 1 33. The method of claim 17 further comprising: 2 (D) identifying one or more test agents that have desirable properties; and 3 (E) producing the one or more test agents as a therapeutic drug. 1 34. The method of claim 17 further comprising removing the engineered 2 cartilage tissue from the semipermeable membrane prior to contacting the engineered cartilage 3 tissue with the test agent.

The method of claim 17 wherein steps (A) and (B) occur in the same well

2 of a multiwell plate.